Supporting Information

Moczek and Rose 10.1073/pnas.0809668106

SI Methods

Western Analysis. Tissue from legs, dorsal abdomen, thoracic horn, and head horn of a single control and *dac*- or *hth*-RNAi individual was dissected and the proteins were extracted by homogenization in 100 μl of extraction buffer (10 mM Tris, pH 7.4, 100 mM KCl, 2.5 mM $MgCl_2$, 2 mM DTT, 0.05% Triton X-100) including protease inhibitors (Calbiochem). Samples were spun for 5 min and the supernatant was assayed for protein concentration by the Bradford method. Samples were mixed with Laemmli sample buffer and heated to 90 °C for 5 min before loading 1–5 μg of protein of each tissue on an SDS/10% polyacrylamide gel. Gel proteins were transferred to nitrocellulose by semidry transfer (TransBlot, Bio-Rad), and poststained with Coomassie blue (reagent gels) and Ponceau S (filters) to assess quality of transfer. Blots were blocked for 3 h at room temperature in 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween 20 (TBST), and incubated overnight at 4 °C in primary rabbit anti-*Tl*dac or rabbit anti-hth (AS1924) at 1:2,000 and 1:4,000, respectively. Anti-α-tubulin DM1A (Sigma) and β-actin (Abcam) were used as loading controls at 1:5,000 for α-tubulin and 1:2,500 for β-actin. Blots were washed 3 times for 20 min each in TBST, incubated in GAR-HRP (Jackson ImmunoResearch) at 1:10,000 for 1 h at room temperature before final washes (3 times for 20 min each in TBST) and placed in ECL substrate (Pierce) and exposed to Kodak BioMax XAR film. This was replicated for a total of 5 individuals for both *hth* and *dac*.

Northern Analysis. *Dll* total RNA was extracted from leg, dorsal abdominal, thoracic horn, and head horn of single 1-day-old *O. taurus* pupae exhibiting severe *Dll* phenotypes by using the RNeasy Mini kit (Qiagen). Samples from 4 individuals of like phenotypes were pooled and 2.5–5.0 μg was run on a 1.2% formaldehyde agarose gel and transferred to BrightStar Plus (Ambion) membrane by using a TransBlot semidry unit (Bio-Rad). Probes were generated by using the BrightStar Psoralen-Biotin kit (Ambion) using a 276-bp fragment of the *OtDll* gene upstream of the homeodomain. Hybridization and washes were done in ULTRAhyb (Ambion) buffer at 68 °C, according to the buffer instructions, and the resulting signal was detected with BrightStar BioDetect according to instructions and exposed to Kodak BioMax XAR film.

1. Kurant E, et al. (1998) Dorsotonals/homothorax, the *Drosophila* homologue of meis1, interacts with *extradenticle* in patterning of the embryonic PNS. *Development* 125:1037–1048.

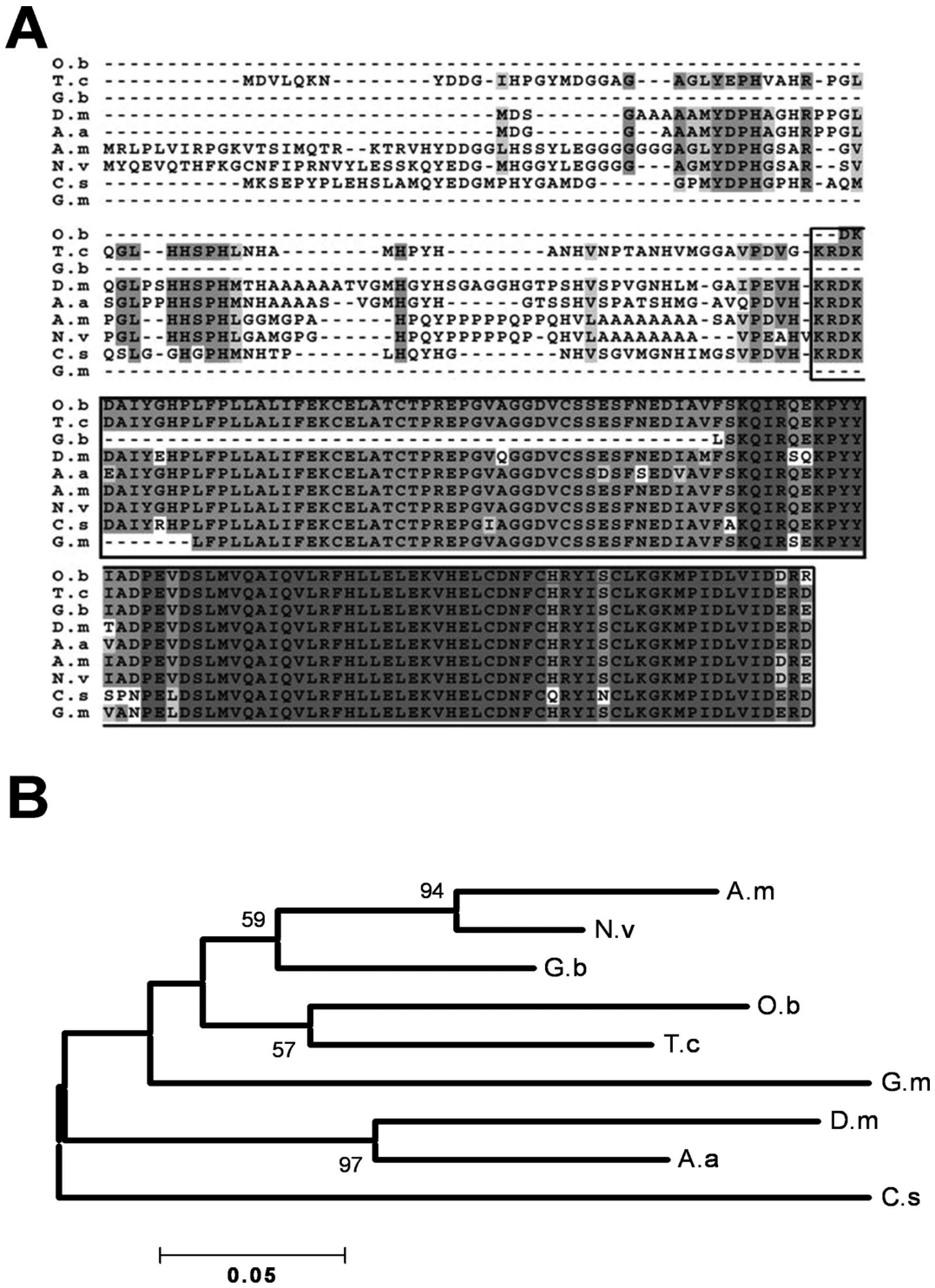

Fig. S1. Sequence analysis of *O. binodis homothorax* (*hth*). (*A*) Multiple sequence alignment of *hth* showing conserved domains of *O. binodis* hth proteins from other arthropods: T.c, *Tribolium castaneum*; G.b, *Gryllus bimaculatus*; D.m, *Drosophila melanogaster*; A.a, *Aedes aegypti*; A.m, *Apis mellifera*; N.v, *Nasonia vitripennis*; C.s, *Cupiennius salei*; G.m, *Glomeris marginata*. Identical residues are in dark gray and residues shared by a majority of species in the alignment are shaded in lighter gray. The conserved MEIS (HM) domain is boxed in black and the region used for RNAi is underlined in red. (*B*) Phylogenetic tree for the multiple alignment shown in *A*. Nucleotide sequences were aligned by using CLUSTALW and the evolutionary relationships were inferred by using the neighbor-joining method with bootstrapping (1,000 iterations). Numbers above or below branches represent the percentage of iterations supporting the branch. The tree is drawn to scale and the evolutionary distances were computed by using the maximum composite likelihood method. The rate variation among sites was modeled with a gamma distribution and all positions with gaps were treated by the complete-deletion method. GenBank accession numbers are as follows: T.c, NP_001034489.1; G.b, BAB79453.1; D.m, AAC47759.1; A.a, XP_001661813; A.m, XP_624460.2; N.v, XP_001601467; C.s, CAD57739.1; G.m, CAD82908.1; and O.b, ACH95872).

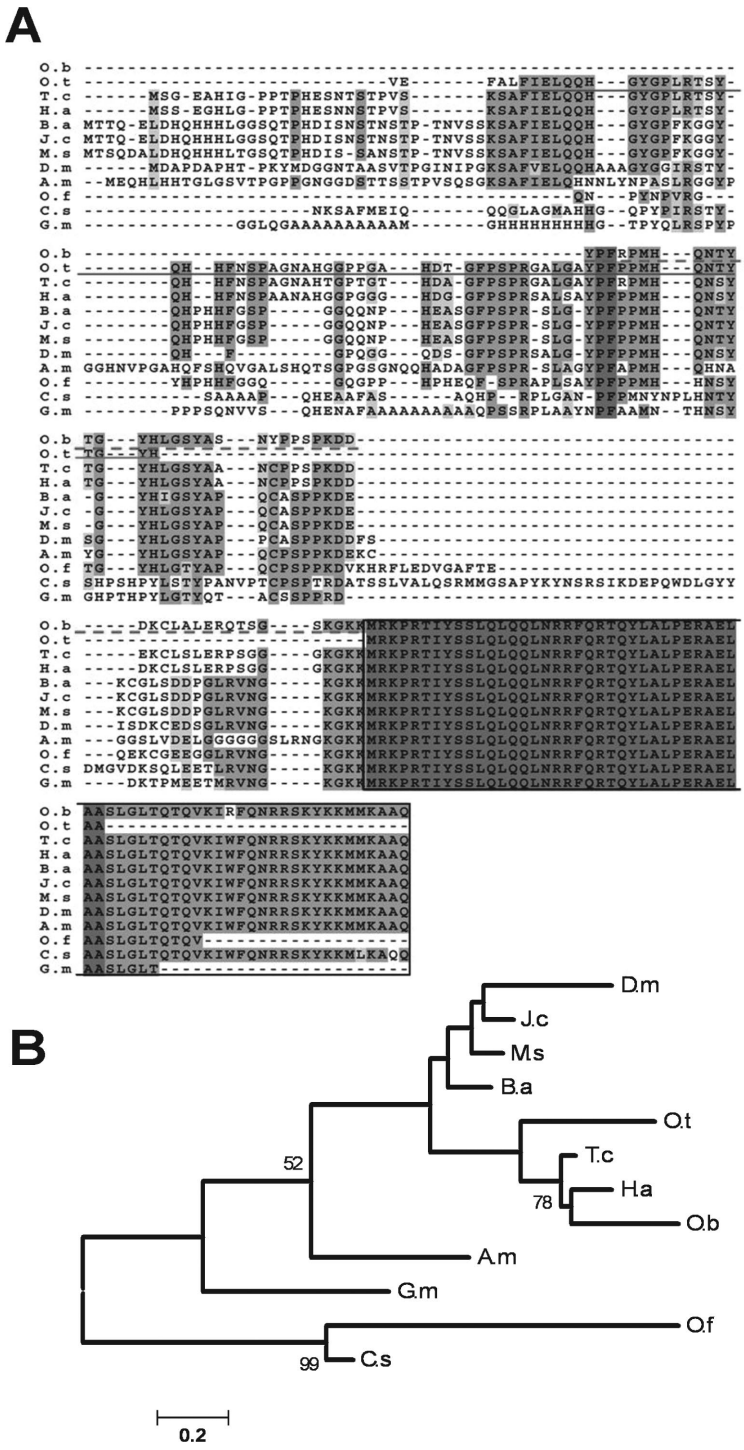

Fig. S2. Sequence analysis of *O. binodis* and *O. taurus Distal-less* (*Dll*). (*A*) Multiple sequence alignment of *Dll* showing conserved domains of *O. binodis* and *O. taurus Dll* and proteins from other arthropods: T.c, *Tribolium castaneum*; H.a, *Harmonia axyridis*; B.a, *Bicyclus anynana*; J.c, *Junonia coenia*; M.s, *Manduca sexta*; D.m, *Drosophila melanogaster*; A.m, *Apis mellifera*; O.f, *Oncopeltus fasciatus*; C.s, *Cupiennius salei*; G.m, *Glomeris marginata*. Identical residues are in dark gray and residues shared by a majority of species in the alignment are shaded in gray. The conserved homeodomain motif is boxed in black. The region used for *O. taurus* RNAi is underlined with a solid red line and the region used for *O. binodis* is underlined with a dashed red line. (*B*) Phylogenetic tree for the multiple alignment shown in *A*. Nucleotide sequences were aligned by using CLUSTALW and the evolutionary relationships were inferred by using the neighbor-joining method with bootstrapping (1,000 iterations). Numbers above or below branches represent the percentage of iterations supporting the branch. The tree is drawn to scale and the evolutionary distances were computed by using the maximum composite likelihood method. The rate variation among sites was modeled with a gamma distribution and all positions with gaps were treated by the complete-deletion method. GenBank accession numbers are as follows: T.c, NP_001034528.2; H.a, BAE78537.1; B.a, AAL69325.1; J.c, AAK97630.1; M.s, AAT39558.1; D.m, AAB24059.1; A.m, XP_001122433.1; O.f, AAS93631.1; C.s, CAC34380; G.m, CAD82905; O.t, ACI41230; and O.b, ACI41229.

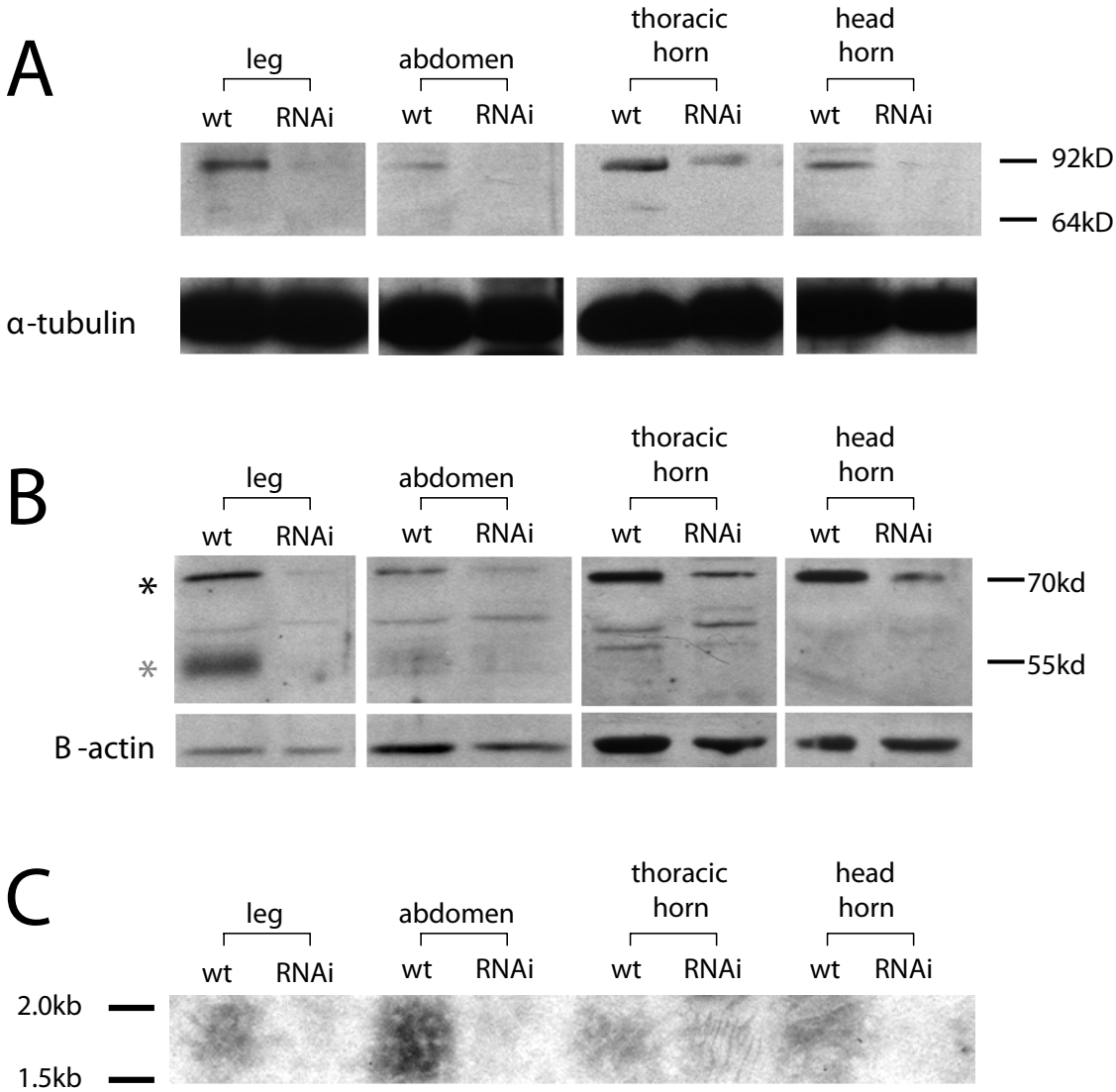

Fig. S3. Validation of RNAi-mediated knockdown of target genes. Shown are Western blot analyses for (*A*) *Otdac*, (*B*) *Othth*, and (*C*) Northern blot analysis for *OtDll* for 4 different body regions: leg, abdomen, thoracic horn, and head horn. For *Othth,* 2 bands were detected. The black star marks a band approximately the size of 65–68 kD reported from extracts of larval *Drosophila* (1). A second band (gray star) of unknown nature is present at ≈55 kD. *dac*RNAi and *hth*RNAi resulted in substantial reductions in gene product across all tissues compared with wild-type. Northern data are consistent with the hypothesis that *Dll* mRNA is also depleted in a nontissue-specific manner across tissues as compared with wild-type. Combined, these results suggest that dsRNA-injections used in the present study degrade their designated targets in a nontissue-dependent manner.

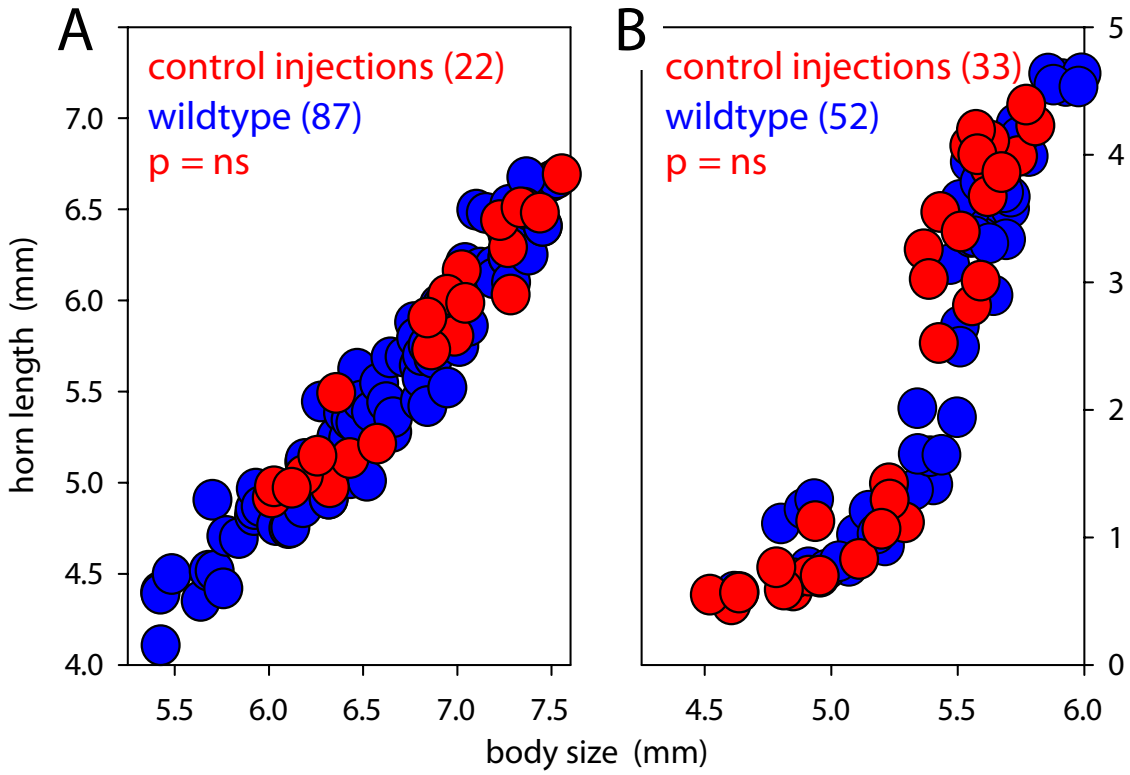

Fig. S4. Scaling relationships between body size and horn length in untreated wild-type animals (blue) and animals injected with 1–5 μg of dsRNA derived from a BlueScript plasmid vector sequence (red) to control for possible artifacts resulting from nucleic acid injections. Data shown are for pupal male *O. binodis* (thoracic horns) (*A*) and *O. taurus* (head horns) (*B*). Sample sizes are given in parentheses. No significant differences were detected.